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# Resident-Invader Phylogenetic Relatedness, Not Resident Phylogenetic Diversity, Controls Community Invasibility

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ABSTRACT: A central goal of invasion biology is to elucidate mechanisms regulating community invasibility. Darwin's naturalization hypothesis, one of the oldest hypotheses in invasion biology, emphasizes the importance of phylogenetic relatedness (PR) between resident and invader species for predicting invasibility. Alternatively, a recent extension of the diversity-invasibility hypothesis predicts that phylogenetic diversity (PD) of resident communities influences invasibility. Neither of these hypotheses has undergone rigorous experimental testing, and the relative contributions of PR and PD to community invasibility are unknown, in part because their effects tend to be confounded with each other. Here we consider both perspectives together by independently manipulating PR and PD in laboratory bacterial assemblages. We found that, although invader abundance decreased significantly as PR increased, it was unaffected by PD. Likewise, we found that resident-invader functional similarity, not functional diversity of resident communities, was a significant predictor of invader abundance. Nevertheless, invader abundance was better predicted by PR than by functional similarity. These results highlight the importance of considering species evolutionary relationships, especially the PR between resident and invader species, for the prediction, prevention, and management of biological invasions.

*Keywords*: bacteria, community invasibility, phylogenetic diversity, phylogenetic relatedness.

## Introduction

The broad ecological and economic consequences of biological invasion have spurred considerable interest among ecologists in exploring mechanisms controlling the invasibility of ecological communities (Vitousek et al. 1996; Tilman 1997; Mack et al. 2000). One aspect of this work has focused on biodiversity, asking the question of whether more diverse communities pose stronger resistance to invaders than their less diverse counterparts. Much of this research has focused on species diversity. Experimental manipulations of species diversity have shown that community invasibility often decreases as the number of species in the community increases (Levine et al. 2002; Fridley et al. 2007). This negative diversity-invasibility pattern can be driven by at least one of two mechanisms: the niche complementarity effect (i.e., niche differentiation among resident species leads to fewer unoccupied niches available for invaders in more species-rich communities) and the sampling effect (i.e., there is a greater chance for more species-rich communities to contain species that strongly resist invasion; Fargione and Tilman 2005).

In light of the existing work linking species diversity and invasion, it is notable that species diversity represents only one component of biodiversity and that it treats each species equally. Species, however, are known to differ in their functional traits and evolutionary history. Other less studied components of biodiversity may better capture these differences in species characteristics within a community. One such component of biodiversity is phylogenetic diversity (PD), which has recently drawn much attention from biologists (Forest et al. 2007; Srivastava et al. 2012). This is facilitated by the increased availability of gene sequences for various organisms and by our improved knowledge of species' functional traits. It has been recognized that species' functional traits, rather than species' identity, determine their contribution to ecosystem functions (Diaz and Cabido 2001; McGill et al. 2006; Ordonez et al. 2010; Eisenhauer et al. 2013). As a result, functional diversity (FD), which better captures functional trait distributions than species diversity, may do a better job predicting ecosystem functions (Wardle et al. 1997; Naeem and Wright 2003; Griffin et al. 2009; Ordonez et al. 2010). Evolutionary histories, however, place a constraint on the differences in functional traits among taxa (Peterson et al. 1999; Martin 2002;

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Petchey and Gaston 2006; Donoghue 2008; Devictor et al. 2010; Flynn et al. 2011), such that PD, which accounts for species evolutionary relationships, may often be a reasonable proxy of FD (Cadotte et al. 2010; Devictor et al. 2010; Flynn et al. 2011; note that PD can also capture unidentified or unmeasurable phenotypic features in addition to common functional traits). Because it may not always be straightforward to identify or measure functionally important traits, PD has been expected to be of much use as a predictor for ecosystem functions (Cavender-Bares et al. 2009; Srivastava et al. 2012). Here we hypothesize that increasing PD of resident communities reduces invasibility (fig. 1*a*). Increasing PD may strengthen the complementarity effect if higher-PD communities are characterized by more diverse traits, translating into more niches being occupied by resident species and fewer opportunities available for invaders. Increasing PD may also result in the sampling effect, if species with particular traits that confer invasion resistance are more frequently present in higher-PD communities. Experimental tests of the role of PD on community invasibility, however, are lacking.

Ecologists have long recognized the potential role of phylogenetic relatedness (PR) between resident and invader species in influencing community invasibility. Darwin (1859) proposed that invasive species would be less successful in communities that contain their close relatives (fig. 1*b*), reasoning that strong competition imposed by resident species on closely related invaders, because of high similarity in their niches, would reduce their success. This naturalization hypothesis is thus related to the sampling effect, where community invasibility is largely determined by certain invasion-resistant species (Wardle 2001). There have been a number of empirical tests of this hypothesis (Ricciardi and Mottiar 2006; Strauss et al. 2006; Proches et al. 2008; Schaefer et al. 2011; Tingley et al. 2011; Allen et al. 2013; Carboni et al. 2013). These tests, which are based almost entirely on observations of nonnative species in their introduced habitats (but see Jiang et al. 2010), have produced mixed results. These conflicting results are difficult to interpret, given the lack of control for confounding factors in observational studies. In particular, in natural communities, changes in PR may be accompanied by changes in PD, making it difficult to separate their effects on invasibility. This problem also plagues those experimental studies that directly manipulated PR or PD, but not both, in relation to invasibility (i.e., Jiang et al. 2010). Given that both PR and PD have the potential to influence community invasibility (fig. 1) and that their effects could be confounded with each other, it is imperative for studies to consider both factors simultaneously.

Here we report on the first experiment, to our knowledge, that independently manipulated PD and PR to evaluate their relative importance for invasibility. In situations where important functional traits are phylogenetically conserved, the independence of PD and PR would allow us to associate a significant PD effect solely with niche complementarity and a significant PR effect with the sampling effect. We assembled resident microbial communities in laboratory microcosms using bacterial species collected from a single source and invaded them with a nonresident bacterial species. We fixed initial resident species richness at a constant level to minimize potential confounding effects of PD and PR from species richness. We hypothesized that both PD and PR would be important in regulating commu-

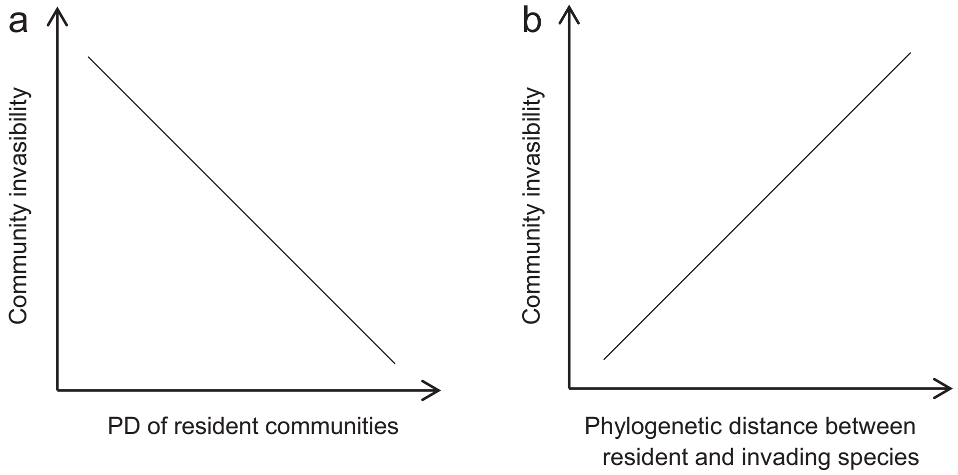

**Figure 1:** Hypothesized effects of phylogenetic diversity (PD; *a*) of resident communities and phylogenetic distance, the inverse of phylogenetic relatedness (*b*), between resident and invading species on community invasibility. Invasibility is expected to decrease as PD increases but increase as phylogenetic distance increases.

nity invasibility, much as both the complementarity and sampling effects contribute to positive relationships between species diversity and community biomass production (e.g., Loreau and Hector 2001). We also measured FD of resident communities and functional similarity (FS) between resident and invading species on the basis of the ability of study bacteria to utilize various organic carbon compounds, and we hypothesized that FD and FS combine to affect community invasibility.

## Material and Methods

### *Bacteria*

Before the experiment, we collected bacterial strains from several freshwater ponds and screened them for suitability as the candidate members of resident communities. Candidate strains must form distinct colonies on agar plates, allowing us to quantify their abundance via plate counts. They also must show a wide range of PR, so that resident communities with different degrees of PD can be constructed. The resultant species pool (see fig. 2) consisted of both gram-positive and gram-negative bacteria, the majority of which were collected from a single pond—Lake Clara Meer in Piedmont Park (Atlanta, GA)—that receives much of its organic carbon from plant leaf litter. We obtained a strain of *Serratia marcescens*, whose colony morphology (with a distinct solid red color) differs from that of all resident species, from Carolina Biological Supply (Burlington, NC) as the invader. The stock culture of each bacterial strain was grown in 0.2% lysogeny broth.

### *Constructing Phylogeny*

We constructed the bacterial phylogeny based on the 16S rRNA gene of each strain in the species pool (including the invader; fig. 2). After sequencing the 16S rRNA, we aligned sequences with the nearest alignment space termination algorithm (DeSantis et al. 2006), selected the best sequence evolution model—TIM3+G with jModelTest (ver. 0.1) using the Akaike information criterion (Guindon and Gascuel 2003; Posada 2008), and assembled the phylogenetic tree with MrBAYES (ver. 3.1.2) using the Bayesian method (Huelsenbeck and Ronquist 2001). Three archaeal species were used as the out-group. We also constructed, with the maximum likelihood method, a phylogenetic tree whose structure was almost identical to that of the Bayesian tree. Pairwise phylogenetic distance matrices derived from the two trees were highly correlated (Mantel test: $r = 0.934$, $P < .001$). The results that were based on the two types of trees were both qualitatively and quantitatively similar, and here we report the Bayesian tree and associated results only.

### *Experimental Design*

Based on the phylogenetic tree, we assembled bacterial communities that differed in three levels of PD (low, intermediate, and high) of resident communities and three levels of PR (low, intermediate, and high) between the resident community and invading species, while fixing the number of species in each resident community at three. All the resident species used in our experiment came from Lake Clara Meer. We acknowledge that laboratory microcosms, including ours, are less complex than natural systems, yet their simplicity facilitates investigations of questions that may be difficult to answer in nature (Jessup et al. 2004). Three-species resident communities also varied widely in PD and PR, maximizing our ability to manipulate them independently of each other. This level of species diversity also allowed the setup of more than one resident community for each PD-PR combination (see table A1, available online), reducing the idiosyncratic influences of individual species and communities on experimental results. Nevertheless, we were unable to find any suitable resident community for the high-PD, low-PR treatment, leaving us an incomplete factorial design (table A1).

Following Faith (1992), we calculated PD of a resident community by summing the lengths of all the intervening branches of the three constituent species with the community. Similarly, we calculated phylogenetic distance (the inverse of PR) between resident and invading species by summing the length of the intervening branches between them on the phylogenetic tree. Both nearest phylogenetic distance (distance between the invader and its closest relative in the resident community) and average phylogenetic distance (average distance between the invader and each species in the resident community) were calculated, and because results based on the two metrics were qualitatively similar, we reported those based on nearest phylogenetic distances only. In addition to the three-species resident communities, we also established monocultures of each resident species used in the experiment and subjected them to the invasion of the same nonresident species. Each of the single- and three-species communities was replicated five and 10 times, respectively.

Each microcosm consisted of one 25-mL loosely capped test tube containing 10 mL of filtered protozoan pellet medium, which supports the growth of bacteria (Jiang 2007; Jiang et al. 2010; Tan et al. 2012). The medium was composed of 0.55 g of crushed protozoan pellet (Carolina Biological Supply) per liter of deionized water. The pellet is made from plant materials and contains a variety of carbon compounds that can be utilized by bacteria. Compared with other commonly used animal protein–originated medium (e.g., nutrient broth and peptone broth), this medium provided our study bacteria with resources more sim-

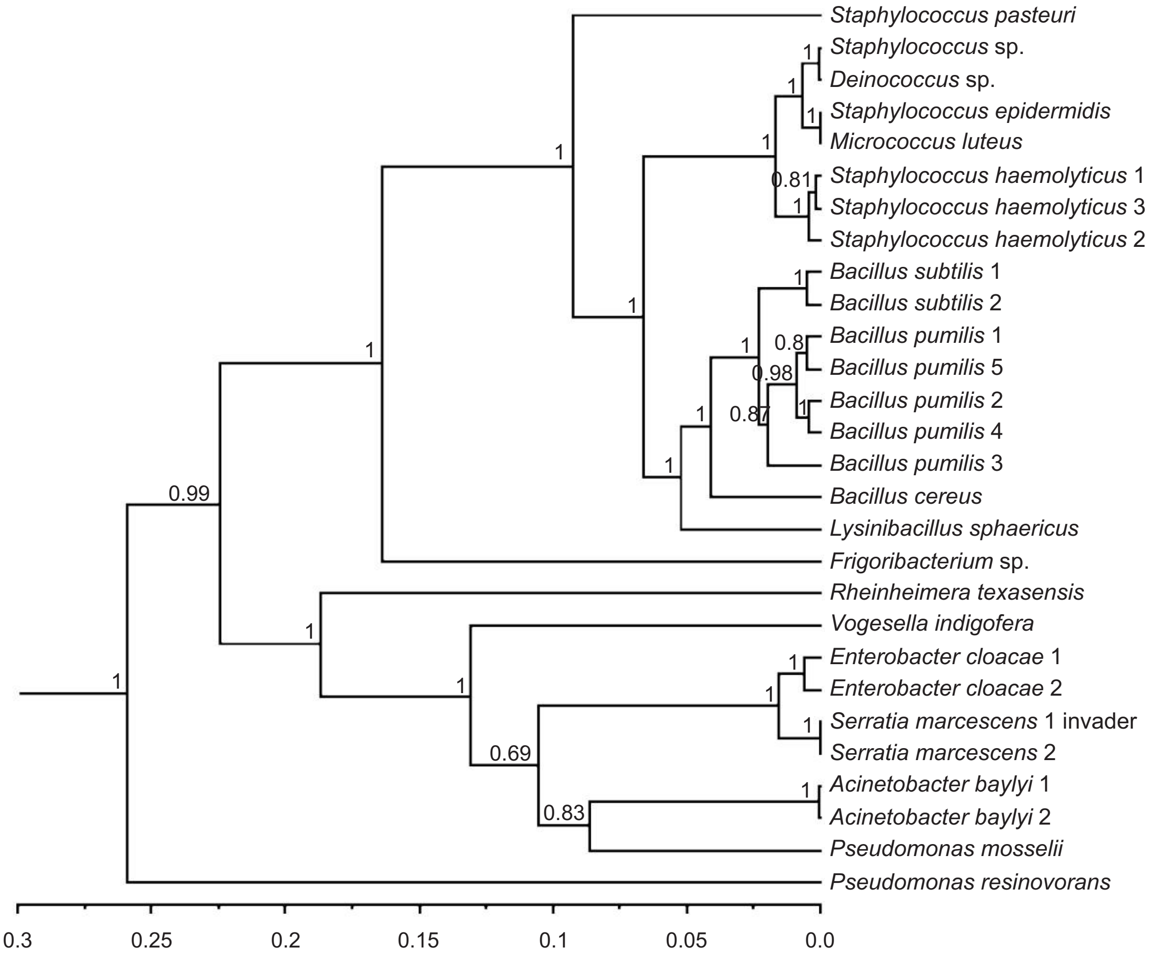

**Figure 2:** Phylogeny of the bacterial species pool based on the 16S rRNA genes, constructed using the Bayesian method. Scores on nodes indicate posterior probability. This phylogenetic tree is deposited in TreeBase: http://purl.org/phylo/treebase/phylows/study/TB2:S17142.

ilar to those in their original environments. This medium was autoclaved in large flasks and filtered afterward to remove insoluble particles. The filtrate was then transferred into experimental microcosms and autoclaved again before the experiment started. Each microcosm also received a wheat seed as the additional carbon source. The wheat seeds were dried, weighed, and autoclaved beforehand. During the experiment, all microcosms were incubated on a shaker at 200 rpm under room temperature (∼22°C).

At the beginning of the experiment (day 0), we introduced $10^4$–$10^5$ individuals of each resident species into microcosms by transferring 3 $\mu$L of their stock cultures with a calibrated aseptic loop. We allowed the resident communities to equilibrate for 41 days before subjecting them to invasion. To determine the realized PD and PR of three-species resident communities at the time of invasion, we destructively sampled half of the microcosms (five replicates of each treatment) on days 40 and 41 to determine resident species composition and abundance. These data were used to calculate realized PD and PR in each sampled microcosm. In addition to the PD described by Faith (1992), realized PD was also calculated with abundance-based metrics as described by Cadotte et al. (2010). As the results based on Faith's PD and other metrics were similar, we presented those based on Faith's PD only. Moreover, we quantified the realized phylogenetic distinctiveness of the invader to resident communities using the quadratic entropy (QE)–based index (Pavoine et al. 2005) as an alternative measure of PR. The QE-based index quantifies the phylogenetic uniqueness of the invader from residents by considering both the branch lengths between the invader and resident species and the topological distribution of these species on the phylogenetic tree. On day 42, we invaded the remaining microcosms (not sampled to avoid contamination) with ∼$10^5$ individuals of the nonresident bacterium *S. marcescens*, introduced in the same way as the resident species. The experiment continued for another 21 days to allow the establishment of invader populations. Final sampling was conducted on day 63 to quantify invader abundance as a common metric of invasibility.

### *Measuring FD and FS*

To directly assess the relevance of functional traits for predicting invasibility, we estimated FD of each resident bacterial community and FS between resident and invading species on the basis of their ability to utilize a variety of carbon substrates on Biolog MicroPlates (Biolog, Hayward, CA). Gram-positive and gram-negative bacterial cultures were inoculated into their corresponding types of MicroPlates, and the results for each of the carbon substrates (positive or negative) were recorded. Our analyses focused on the 55 substrates that gram-positive and gram-negative MicroPlates share in common. To reduce data dimension, we conducted a principal component analysis of the 55 traits and extracted the first four principal components that accounted for 51% of the variance in the data. We then calculated the realized functional distance (the inverse of FS) between resident and invader species as their Euclidean distance in the four-dimensional space, and we realized FD of resident communities as the summed Euclidean distance among the resident bacterial species in the four-dimensional space. Both nearest and average realized functional distances between the invader and resident communities were calculated, and analyses based on the two metrics produce similar results. We thus reported findings based on nearest distances only. We also calculated realized functional distances between invader and resident species and FD of resident communities on the basis of only those traits that exhibited significant phylogenetic signal (see below for tests of phylogenetic signal), using the same methods described above.

### *Data Analysis*

We used a two-way ANOVA to assess the effects of phylogenetic diversity (PD) and phylogenetic relatedness (PR) on community invasibility, with invader abundance as the dependent variable and initial PD and phylogenetic distance as the class variables. Type IV sums of squares were used to account for the unbalanced treatment combinations (Shaw and Mitchell-Olds 1993). Tukey's highly significant difference [HSD] post hoc test was conducted after the detection of significant treatment effects. We performed simple linear regressions modeling invader abundance as a function of realized PD, realized phylogenetic distance, realized FD, and realized functional distance separately. The realized diversity and distance values of each treatment were calculated as the average of the five replicate microcosms destructively sampled before invasion, based on the assumption that the remaining five replicate microcosms would hold comparable values before invasion (see also Jiang et al. 2010). We then performed a multiple regression that considered all explanatory variables together, with the backward selection procedure used to identify the variables best explaining invasibility. Bacterial abundance data were log transformed before statistical analyses to improve normality.

We tested for significance of phylogenetic signal of the measured functional traits in two ways. First, we considered all traits together with a Mantel test assessing the association between bacterial phylogenetic and functional distances, with distance matrices permutated 9,999 times. Second, we tested for the phylogenetic signal of each trait individually with Blomberg's (2003) $K$ statistic, with 9,999 permutations. We also used false discovery rate correction to control for type I error for the 55 individual tests, following Benjamini and Hochberg (1995) and Storey et al. (2004). All statistical analyses were performed in SPSS 16 (SPSS, Chicago, IL) with the exception of the QE-based index and Blomberg's $K$ (Blomberg et al. 2003), which were calculated using the originality function in the R package ade4 (Dray and Dufour 2007) and the multiPhylosignal function in the R package picante (Kimbel et al. 2010), respectively.

## Results

The nonresident invader successfully established its populations in all experimental microcosms. Its abundance, however, varied substantially among the experimental treatments (fig. 3). ANOVA revealed a highly significant effect of PR ($F_{2,82} = 35.667$, $P < .001$) and a marginally significant effect of PD ($F_{2,82} = 3.168$, $P = .047$) on invader abundance; changes in PD did not alter the effect of PR on invasibility (PD × PR term in ANOVA: $F_{3,82} = 1.117$, $P = .347$). The significant PR effect was largely driven by lower invader abundance in the high PR (i.e., low phylogenetic distance) treatment than in the low and intermediate PR (i.e., large and intermediate phylogenetic distance) treatments (Tukey HSD test, $P < .001$). Tukey's HSD test, however, did not detect significant difference in invader abundance among the three PD treatments ($P > .25$). Data underlying our figures are deposited in the Dryad Digital Repository: http://dx.doi.org/10.5061/dryad.9sj18 (Tan et al. 2015).

Linear regressions showed that invader abundance in multiple-species resident communities was unaffected by realized PD ($R^2 = 0.024$, $P = .148$; fig. 4*a*). Invader abundance, however, increased as phylogenetic distance between the resident and invader species increased (i.e., decreasing

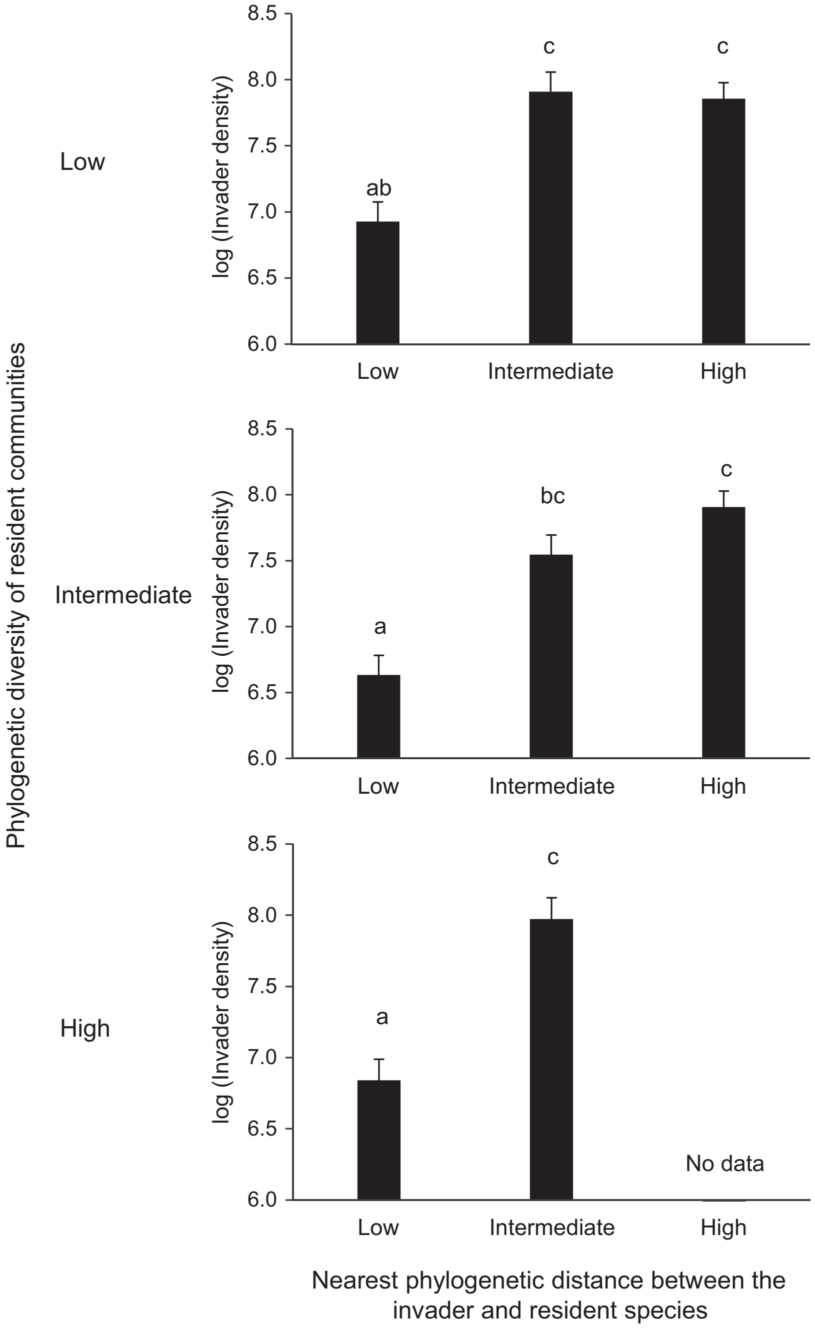

**Figure 3:** Population densities of the invader (*Serratia marcescens*) in three-species resident communities with different levels of phylogenetic diversity (PD) and nearest phylogenetic distance (the inverse of phylogenetic relatedness) between resident and invading species. Values are mean ± standard error, with population densities measured as colony-forming units per milliliter and $\log_{10}(x+1)$ transformed before analysis. Treatments sharing the same letters do not differ from each other according to Tukey's highly significant difference tests. Note the absence of the high PD–high phylogenetic distance treatment.

with PR; $R^2 = 0.560$, $P < .001$; fig. 4*b*). Note that species extinction occurred in some of the resident communities without causing realized PR and PD to covary ($R^2 = 0.098$, $P = .357$). Invader abundance in multiple-species resident communities also increased as the invader's phylogenetic distinctiveness, characterized as the QE-based index, increased ($R^2 = 0.125$, $P = .001$; fig. 4*c*). Phylogenetic distance was also a significant predictor of invader abun-

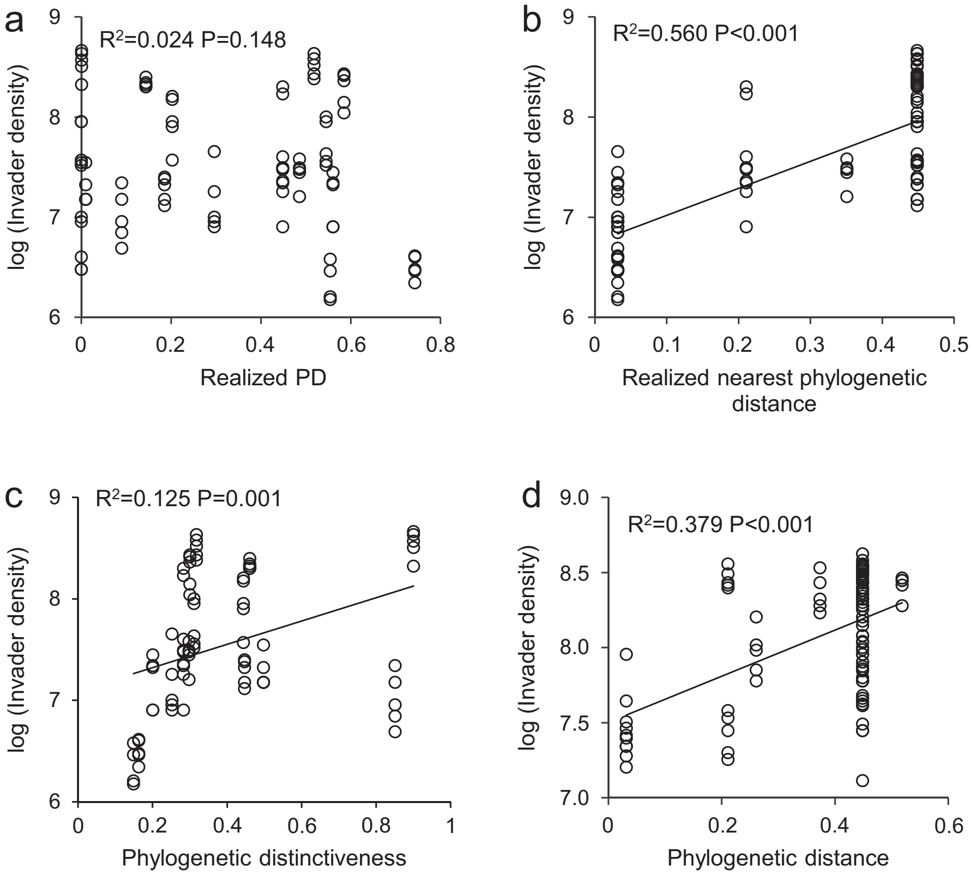

**Figure 4:** Relationships between invader population density and (*a*) realized phylogenetic diversity (PD) of three-species resident communities; (*b*) realized nearest phylogenetic distance (the inverse of phylogenetic relatedness [PR]) between the invader and three-species resident communities; (*c*) realized phylogenetic distinctiveness of the invader to three-species resident communities; and (*d*) realized phylogenetic distance (the inverse of PR) between the invader and single-species resident communities. Realized diversity, distinctiveness, and distance values were calculated on the basis of data collected immediately before invasion. Data are plotted with significant linear regression lines. Invader population densities are given in colony-forming units per milliliter and were $\log_{10}(x + 1)$ transformed before analysis.

dance in single-species resident communities ($R^2 = 0.379$, $P < .001$; fig. 4*d*).

When all the 55 measured traits were considered together, our study bacteria exhibited significant phylogenetic signal (Mantel test, $r = 0.2341$, $P = .0041$). When examined individually, 8 (15%) of the 55 traits, including D-trehalose ($K = 1.250$, $P = .009$), $\alpha$-D-glucose ($K = 0.472$, $P = .015$), D-melibiose ($K = 1.032$, $P = .022$), D-fructose ($K = 0.355$, $P = .031$), gentiobiose ($K = 0.305$, $P = .034$), $\beta$-methyl-D-glucoside ($K = 0.481$, $P = .035$), $\alpha$-D-glucose-1-phosphate ($K = 1.016$, $P = .037$), and D,L,$\alpha$-glycerol phosphate ($K = 0.255$, $P = .046$), exhibited significant phylogenetic signal. None of the traits, however, remained significant after correction for the false discovery rate.

When considering the principal components extracted from all traits, we found that FD did not affect invader abundance in multispecies resident communities (fig. 5*a*; $R^2 < 0.001$, $P = .922$). Invader abundance, however, increased with functional distance between the invading and resident species in both multiple-species (fig. 5*b*; $R^2 = 0.180$, $P < .001$) and single-species resident communities (fig. 6*a*; $R^2 = 0.048$, $P = .029$). Similar patterns also emerged when functional distance (figs. 5*d*, 6*b*) and FD (fig. 5*c*) were calculated using eight traits with significant phylogenetic signal. Single-trait-based functional distance also showed a positive relationship with invader abundance in single-species resident communities for three of the eight traits ($\alpha$-D-glucose-1-phosphate [$R^2 = 0.245$, $P < .001$], D,L,$\alpha$-glycerol phosphate [$R^2 = 0.130$, $P < .001$], and D-melibiose [$R^2 = 0.110$, $P < .001$]). The multiple regression model retained resident-invader phylogenetic distance, among all the phylogenetic and functional metrics, as the best pre-

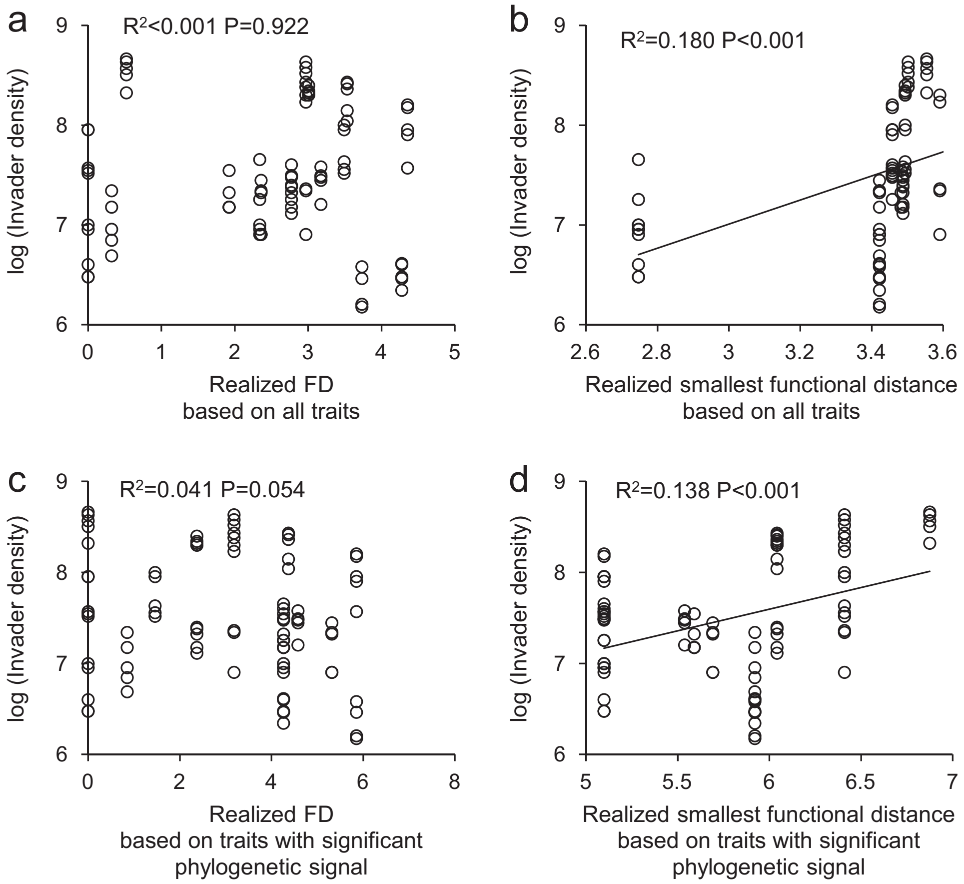

**Figure 5:** Relationships between invader population density and realized functional diversity (FD) of three-species resident communities (*a*, *c*) and realized smallest functional distance (the inverse of functional similarity) between the invader and three-species resident communities (*b*, *d*). FD and functional distance were calculated on the basis of the principal components extracted from the 55 measured traits (*a*, *b*) and on the eight traits showing significant phylogenetic signal (*c*, *d*). Realized FD and distance values were calculated on the basis of data collected immediately before invasion. Data are plotted with significant linear regression lines. Invader population densities are given in colony-forming units per milliliter and were $\log_{10}(x + 1)$ transformed before analysis.

dictor for invader abundance in both multiple- and single-resident communities.

## Discussion

Our study demonstrates a more important role of resident-invader phylogenetic relatedness than resident phylogenetic diversity in determining invasibility. We found that increasing resident-invader phylogenetic relatedness resulted in reduced invader abundance in both multispecies and single-species resident communities, independent of the effects of phylogenetic diversity. There has been substantial recent interest in evaluating Darwin's naturalization hypothesis in natural assemblages of various organisms. For example, Duncan and Williams (2002) found that exotic seed plant species with their congeneric native species present in New Zealand were more likely to naturalize in the country, a pattern at odds with the hypothesis. By contrast, Strauss et al. (2006) found that the invasiveness of exotic grass species was negatively associated with their phylogenetic relatedness to native grass species in California, a finding consistent with the hypothesis. These conflicting results, which were based on observations of natural communities (e.g., Duncan and Williams 2002; Lambdon and Hulme 2006; Ricciardi and Mottiar 2006; Strauss et al. 2006; Schaefer et al. 2011), underscored the need for rigorous tests of Darwin's naturalization hypothesis via experimentation. Jiang et al. (2010) conducted such an experiment using bacteria and reported results consistent with Darwin's prediction, but this study did not account for the fact that relatedness and diversity effects were confounded with each other. This is not an issue in the experiment reported here, in which phylogenetic relatedness and diversity were independently manipulated. The significant effect of phyloge-

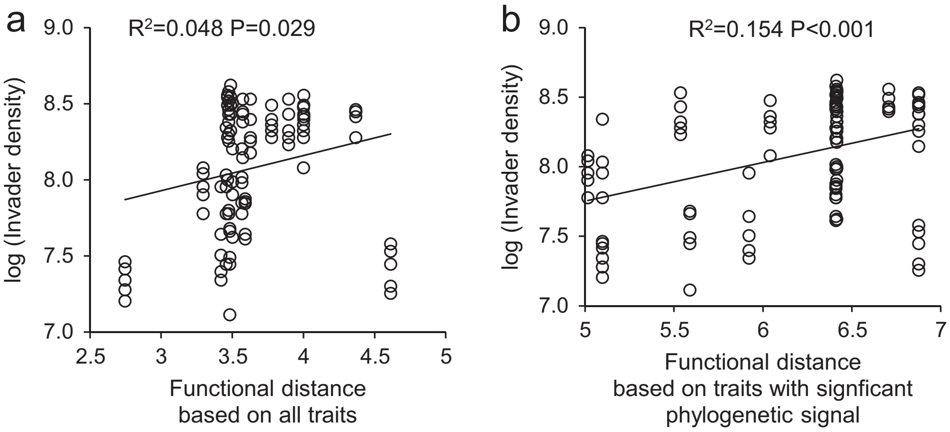

**Figure 6:** Relationships between invader population density and functional distance (the inverse of functional similarity) between the invader and single-species resident communities. Functional distances were calculated on the basis of the principal components extracted from the 55 measured traits (*a*) and on eight traits showing significant phylogenetic signal (*b*). Data are plotted with significant linear regression lines. Invader population densities are given in colony-forming units per milliliter and were $\log_{10}(x + 1)$ transformed before analysis.

netic relatedness in our experiment points to the importance of the presence of particular species for resisting biological invasions; that is, resident communities are more resistant to invasion because they contain species that are more closely related to the invader (i.e., the sampling effect). In particular, the resident communities characterized by the highest phylogenetic relatedness, containing different *Enterobacter cloacae* strains, were most closely related to the invader (fig. 2) and least receptive to invasion (fig. A1, available online). Overall, our study provided the first unambiguous experimental evidence for Darwin's naturalization hypothesis, supporting the utility of phylogenetic relatedness between resident and invader species for predicting community invasibility.

In contrast to the strong effect of resident-invader phylogenetic relatedness, we found little effect of phylogenetic diversity of resident communities on invasibility. This surprising result is at odds with the traditional view that more diverse communities are more resistant to invasion (Elton 1958). It also contrasts with the result of a recent observational study that higher phylogenetic diversity plant communities in the Netherlands were less receptive to alien plant species (Gerhold et al. 2011). This apparent discrepancy, however, may potentially be explained by the fact that our study considered the independent contributions of resident-invader phylogenetic relatedness and resident phylogenetic diversity on community invasibility, whereas Gerhold et al. (2011) focused on resident phylogenetic diversity, whose effect may not be completely separated from that of resident-invader phylogenetic relatedness. It is likely that higher-diversity plant communities in Gerhold et al. (2011) were more resistant to invasion because they were more likely to contain close relatives of the invaders, which is an idea worthy of future exploration. Also note that many empirical studies (including both observations and experiments) have examined the relationship between resident species diversity and community invasibility without taking a phylogenetic perspective (Fridley et al. 2007). Although beyond the scope of our study, results from these previous studies could be mined (e.g., using meta-analyses) to explore how resident phylogenetic diversity and resident-invader phylogenetic relatedness influence invasibility. Nevertheless, analyses of this kind would still not be able to identify independent contributions of the two factors as in our study. A second possibility is that niche complementary among resident species (corresponding to significant phylogenetic diversity effects) was simply more important in the study of Gerhold et al. (2011) than in our study. In our experiment, the sampling effect (corresponding to significant phylogenetic relatedness effects) dominated, with pairwise species interactions between the invader and its closest relative in the resident community largely determining its invasibility, and the niche complementarity among resident species was unimportant. This variation in the relative importance of mechanisms is common among biodiversity and ecosystem function studies, including those that examined other metrics of biodiversity in relation to biological invasions (e.g., Stachowicz et al. 1999 vs. Arenas et al. 2006). Mechanistically, the lack of complementarity among resident species in resisting invasion in our study may simply reflect the relatively large phylogenetic scale concerning our study bacteria, such that resident species not closely related to the invader posed little resistance to invasion (fig. A1) due to their substantial niche difference. A third possibility that could potentially explain this discrepancy is that the strength of the relationship with phylogenetic diversity of resident communities may differ for different invasibility metrics. Invader establishment success, the metric used in Gerhold et al. (2011), may be tightly linked with phylogenetic diversity, because higher-diversity communities would have less unoccupied niche space left for invaders, resulting in their higher establishment failure. On the other hand, invader abundance, the metric used in our study, may be closely associated with phylogenetic relatedness, because invaders may not be able to attain large abundance if their optimal niches are occupied by their closely related resident species. A fourth possibility associated with the lack of the effect of phylogenetic diversity on invasibility is that the lateral transfer of genes controlling ecologically important traits among bacteria may reduce the degree of trait conservatism (Boucher et al. 2003), making phylogenetic diversity, which is based on evolutionarily conservative genetic markers (e.g., 16S rRNA in our study), a less effective proxy of niche complementarity in bacterial communities. However, current evidence indicates that many bacterial functional traits exhibit phylogenetic signal despite lateral gene transfer (Martiny et al. 2013). Some of the bacterial traits examined in our experiment were also phylogenetically conserved (although the phylogenetic signal of these traits were not significant after adjusting for false discovery rate), and the whole set of traits was conserved when considered together. The significant effect of phylogenetic relatedness on invader abundance is also indicative of the conservatism of important functional traits for resident-invader interactions that determined invader abundance.

The patterns based on functional metrics are qualitatively similar to those based on phylogenetic metrics. Specifically, functional similarity between resident and invading species, but not functional diversity of resident communities, influenced invader abundance. This result is robust to whether our analyses were based on all the functional traits measured in our study or only those with significant phylogenetic signal. Functional similarity based on three single phylogenetically conserved traits also influenced invader abundance. Note that a number of studies have demonstrated the role of resident-invader functional similarity (e.g., Fargione et al. 2003; Ordonez et al. 2010; Eisenhauer et al. 2013) or functional diversity of resident communities (e.g., Dukes 2001; Britton-Simmons 2006; Moseman et al. 2009; Vaz-Pinto et al. 2012; Byun et al. 2013) for predicting invader success. In those studies that focused on functional diversity, the effects of functional diversity and similarity were presumably confounded with each other; it remains to be seen whether findings similar to ours would be obtained if these studies also take resident-invader functional similarity into account. On the other hand, it is important to note that resident-invader phylogenetic relatedness explained substantially more variation in invader abundance than resident-invader functional similarity in our experiment. This result suggests that at least some of the important functional traits for resident-invader interactions, although not measured in our experiment, are phylogenetically conserved. The greater predictive power of phylogenetic relatedness demonstrated here supports the idea that species phylogenetic knowledge would be particularly useful for predicting ecosystem functions where important species traits may not be identified or measured (Srivastava et al. 2012).

Although few experimental tests of Darwin's naturalization hypothesis exist, several recent experiments have examined the related hypothesis of Darwin (1859) that stronger competition exists between more closely related species (termed the phylogenetic limiting similarity hypothesis by Violle et al. 2011). For example, Violle et al. (2011) showed that both the frequency and tempo of competitive exclusion increased with the phylogenetic relatedness of ciliated protist species competing for bacterial resources. Burns and Strauss (2011) reported that native plant species in the Bodega Bay Marine Reserve of California tended to perform better when grown in the neighborhood of distant relatives. On the other hand, the outcome of competitive interactions has been found to be unrelated to phylogeny in communities of freshwater green algae (Narwani et al. 2013), herbivorous marine amphipods (Best et al. 2013), and annual plants (Godoy et al. 2014). These conflicting results reflect the variation among the different groups of study organisms in the degree of conservatism of important competition-related traits. The limited number of studies, however, makes it difficult to draw any general conclusion on the relationships between phylogeny and competition. It remains to be seen how the importance of resident phylogenetic diversity and resident-invader phylogenetic relatedness for invasibility varies across different taxonomic groups.

Several caveats of our experiments are worth noting here. First, only one invader species was used to challenge resident communities, raising the possibility that our findings may be influenced by idiosyncratic characteristics of the species. This concern is somewhat alleviated by the fact that, within each PD-PR combination treatment, the invader faced multiple, different resident communities, which reduced the impacts of individual species (including both resident and invader species) on invasion success. Nevertheless, the idiosyncratic effects of the invader traits cannot be completely excluded, and future studies should include multiple invader species to evaluate the robustness of our results. Second, bacterial functional traits were measured on the basis of their ability to exploit different carbon sources in our experiment. We focused on these traits because of their direct relevance for competition in our experiment. It remains to be seen whether considering other types of bacterial traits, such as cell size, capsule formation, and swimming behavior (Matz et al. 2002; Mulder et al. 2005), would improve the ability of functional similarity and diversity to predict invasibility. Third, in our experiment, no resident communities, even those with the invader's closest relatives, completely resisted invasion. One potential contributing factor is that no resident species belonged to the same genus as the invader, a consequence of our experimental design that guaranteed the independence of phylogenetic diversity and phylogenetic relatedness and the requirement that species exhibiting similar colony morphology could not be used together. A complete resistance to invasion may be more likely to emerge in resident communities if they contain species that are more closely related to invaders (e.g., Jiang et al. 2010). Another contributing factor for invader establishment success across all experimental microcosms is the superior competitive ability of the invader (Jiang 2007; Tan et al. 2012). In their evolutionary imbalance hypothesis, Fridley and Sax (2014) suggested that nonresident species experiencing stronger competitive selection over longer timescales while maintaining larger populations in their original habits would be more invasive. The invader used in our experiment, *Serratia marcescens*, which is ubiquitous across varieties of ecosystems, appears to meet the conditions for successful invaders depicted in this hypothesis. Overall, our result is consistent with the general finding that biological invasions cannot be completely repelled in most ecological communities (Levine et al. 2004).

In conclusion, our experimental results did not lend support to our original hypothesis that phylogenetic diversity of resident communities and phylogenetic relatedness between resident and invading species combine to regulate community invasibility. Rather, invader abundance was suppressed by the presence of close relatives but not influenced by phylogenetic diversity of resident communities. This finding provides unequivocal support for Darwin's naturalization hypothesis, reinforcing the importance of knowledge of phylogenetic relationships between invading and resident species for predicting invasibility. In addition, our experiment showed that trait similarity between resident and invading species was more important than trait diversity of resident communities in modulating invader success. Importantly, resident-invader phylogenetic relatedness is a much better predictor of invader success than their functional similarity in our experiment, demonstrating the utility of species phylogenetic information for improving our understanding of regulatory mechanisms of biological invasions. Our study also highlights the need to identify and measure functionally important traits to better our ability to predict invasion resistance and other ecosystem functions. Until a thorough understanding of species traits relevant for various ecosystem functions is attained, combining species phylogenetic information with knowledge of known functional traits may be our best tool for predicting the ecological consequences of changes in biodiversity.

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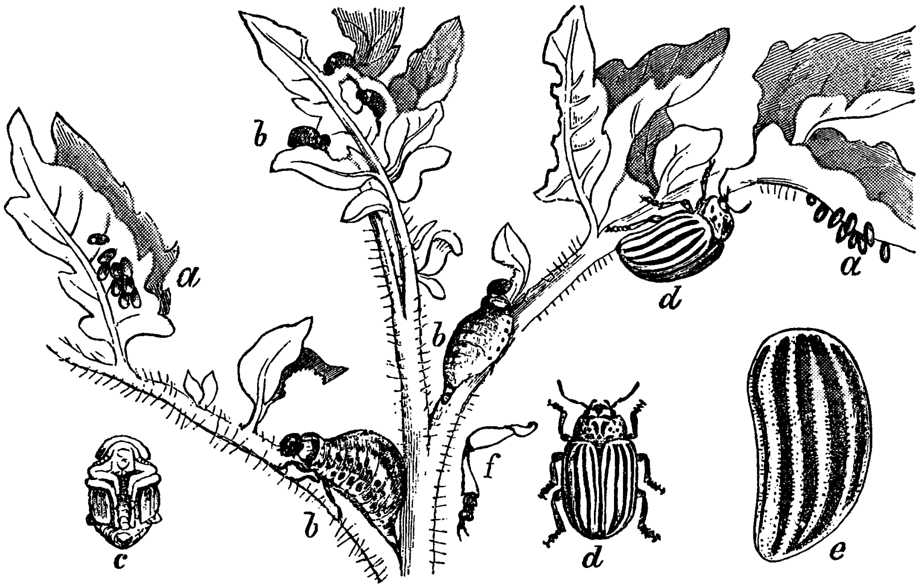

"Of the several distinct species of potato bugs, the Colorado Beetle (*Doryphora 10-lineata* Say [. . .]; *a*, eggs; *b*, young and fully grown larvæ; *c*, pupa; *d*, beetle; *e*, left wing cover, magnified; *f*, leg, magnified) has chiefly attracted attention at the West during the few years past. It has been very destructive, hence anything bearing upon its habits is interesting to farmers. Last year they were more numerous in Illinois than at any other time. Whole acres were entirely destroyed by them." From "Insects Injurious to the Potato" by Henry Shimer (*The American Naturalist*, 1869, 3:91–99).